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THESIS OF DISSERTATION FOR CANDIDATE DEGREE

ORGANIZATION OF THE PHOTOSYNTHETIC APPARATUS IN MODIFIED CHLOROPLAST MEMBRANES

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The photosynthetic light-energy utilization is a vital autotrophic process which determines the overall productivity of the biosphere. This complicated reaction sequence takes place in the chloroplasts of plants, which possess an organized internal membrane system, containing the essential pigment-protein complexes and the components of the electron transport chain. The proper organization of the individual components as well as their assembly into structurally intact and functionally competent units is controlled by exogenous and endogenous factors. Studying the relationship between the structure and composition of photosynthetic membranes and their functional properties can significantly improve our present-day understanding about the details of the photosynthetic processes and can also contribute to the understanding of structure-function relationship in membranes in a more general sense.

Photosynthetic membranes possess some unique features as compared to other membrane systems of animal and plant origin. As concerns their lipid composition, the high proportion of galactolipids is of note, which frequently contain poly-unsaturated fatty acids (e. g. linoleic and linolenic acids). This specific lipid pattern and fatty-acid composition are the main determinants of the high fluidity level of chloroplast membranes. Membrane fluidity, or more precisely the dynamic properties of membranes have been shown to be inherently involved in important cellular processes, but in this respect little is known about the functional significance of the specific fatty acid pattern of chloroplast membranes.

Chloroplast membranes are known to contain different pigments other than chlorophylls, e. g. carotenes and xanthophylls. Their primary role in photosynthesis was assumed to be accessory pigments, which also have effective protective action against photodecomposition of photosynthetic membranes. Recently, however, it is becoming evident that these pigments also play an important role in the assembly and proper organization of pigment-protein complexes, the details of which have not been clucidated so far.

A possible way of studying these and similar problems is to bring about modifications in the usual stoichiometry of the respective components of the membrane and to study the functional consequences of the modifications. The use of specific chemical agents for this reason seemed to us a reasonable approach.

The experimental work presented in this dissertation aimed at studying

1. the structural and functional aspects of the photosynthetic apparatus in chloroplast membranes where

a) the fatty acid composition had been modified;

b) the carotenoid pigment composition had been modified.

2. the interrelationship between linolenic acid content of chloroplast membrane lipids and the photosynthetic activity of chloroplasts and leaves.

3. the direct action on photosynthesis of the chemicals that were used for causing compositional changes.

EXPERIMENTAL DETAILS

The experiments were carried out with barley (*Hordeum vulgare* L., cv. Horpácsi kétsoros) grown for 6 days under controlled laboratory conditions. The chemicals used for modifying the composition of chloroplast membranes were: *cerulenin*, an antibiotic which is an inhibitor of the do novo fatty acid biosynthesis; and two pyridazinone compounds: *SAN 6706* and *SAN 9785*, the former being a specific inhibitor of carotene biosynthesis, whereas the latter inhibits the formation of linolenic acid. The action of chemicals was studied in different experimental systems: during greening of etiolated leaves as well as during treatment of plants from the onset of germination. The direct action of the chemicals was sudied by treating fully developed green leaves.

The fatty acids were separated and analysed by gas-liquid chromatography following thin-layer chromatographic separation of lipids. Pigment content was determined by conventional photometric methods. The fine structure of chloroplasts was studied by electron microscopy. The greening of etiolated leaves was traced by low-temperature fluorescence measurements on intact leaves. In vivo photosynthetic activity of intact leaves was studied by means of fluorescence induction and $^{14}CO_2$-fixation measurements. In vitro photosynthetic activity of isolated chloroplasts was studied by polarographic measurements of oxygen uptake or evolution in the presence of various electron donor/acceptor systems. A rough estimation for the tightness of coupling between electron flow and photophosphorylation was achieved by the use of uncouplers and phosphorylation co-factors. The qualitative pattern of chlorophyll-protein complexes was studied by gel-electrophoretic separation of the polypeptides after SDS-solubilization of chloroplast membranes.

NEW EXPERIMENTAL RESULTS

— when chloroplastic fatty acid biosynthesis is inhibited by the antibiotic cerulenin, the overall greening process is slowed down, the appearances of the two photosystems and of the electron transport activity are delayed. Nevertheless,

cerulenin treatment did not cause complete absence or inactivation of either of the photosystems;

— to the best of our knowledge, our group was the first to demonstrate that cerulenin is a potent inhibitor of de novo fatty acid biosynthesis in chloroplasts. Furthermore, a specific action of cerulenin was observed in the fatty acid compositions of MGDG and phosphatidyl choline;

— when linolenic acid biosynthesis is inhibited in the developing chloroplasts by SAN 9785, a reduction in the amount of the chlorophyll-protein complex(es) of photosystem-I was observed. Although both photosystems were found to be competent in vitro, the in vivo photosynthesis of the leaves was considerably reduced. It is concluded that the reduced linolenic acid content could contribute to the reduction of the photosynthesis, since;

— a strong correlation between the linolenic acid content of chloroplast membrane lipids and the photosynthetic activity of leaves and of the chloroplasts isolated from them was established. In particular, the activity of photosystem-II and the tightness of coupling showed strong correlations with the actual linolenic acid content;

— when carotene biosynthesis is inhibited in developing chloroplasts by SAN 6706, no photosystem-II activity was detected with an apparent lack of the chlorophyll-protein complex of photosystem-II. The lack of carotene pigments, however, did not lead to the disappearance of xanthophylls, which, together with the remaining chlorophylls, could exert important stabilizing role on the light-harvesting chlorophyll-protein complex;

— photosystem-I was found to be competent both in linolenic acidand carotene-deficient chloroplasts, but its chlorophyll-protein complex(es) exhibited different spectroscopic characteristics when isolated from the membrane, suggesting that the complexes developed in the treated leaves possessed reduced structural intactness;

— a strong correlation was observed between the $(F_m—F_i)/F_m$ ratio, calculated from the fast-fluorescence induction curves of leaves, and the relative area above the fluorescence induction curves. This means that the $(F_m—F_i)/F_m$ quantity can serve as a reliable indicator of the rate of electron transport between Q and the plastoquinone pool, therefore, its use in quick tests is recommended.

The experimental data obtained during the studies are mainly concerning with basic science, since our primary interest was to study the organization of photosynthetic membranes and to seek for interrelations between structure and function in chloroplast membranes. We hope, however, that the demonstrated actions of the respective chemicals could initiate further studies for their potential applicability in terms of plant protective strategies.